Journal of Medical Virology 61:398–402 (2000)

Response of Pre-Core Mutant Chronic Hepatitis B Infection to Lamivudine

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The proportion of chronic liver disease associated with the pre-core mutant of hepatitis B virus (HBV) infection is increasing, particularly in Mediterranean Europe and in Asia. The pre-core mutant HBV is unable to produce hepatitis B e antigen (HBeAg), so that patients with this variant do not present with HBV characterised by HBeAg in the serum. Pre-core mutant chronic hepatitis B infection usually proceeds to serious liver disease. Wild-type HBV infection may be mild and respond relatively well to interferon (IFN) alpha therapy, but IFN alpha is not an effective therapeutic option in pre-core mutant hepatitis B infection and new therapeutic options are needed. Clinical data show that lamivudine is an effective treatment for patients with pre-core mutant hepatitis B. There is profound suppression of HBV replication and improvement in indicators of liver disease in most patients. In conclusion, lamivudine is suitable for treatment of a wide range of patients with chronic hepatitis B, including those with pre-core mutant HBV infection. ***J. Med. Virol. 61: 398–402, 2000.***

KEY WORDS: epidemiology; YMDD variant; nucleoside analogue; treatment; liver histology

INTRODUCTION

The normal course of infection with hepatitis B virus (HBV), after a period of immune tolerance in those infected at birth or early childhood, is for the immune response to eliminate the free, replicating form of the virus [Dusheiko, 1999]. This elimination is associated with cell-mediated immune responses and antibody responses directed against hepatitis B e antigen (HBeAg), followed by seroconversion, during which HBeAg and HBV DNA become undetectable and antibodies (anti-HBe) appear. In some patients, hepatitis continues after the appearance of anti-HBe and the disappearance of HBeAg, and some patients are anti-HBe-positive throughout the entire course of their disease. This continuation of disease is associated with the presence of a variant form of HBV harbouring a mutation in the pre-core region that prevents the virus from forming HBeAg [Brunetto et al., 1989; Carman et al., 1989; Chu and Liaw, 1997]. Infection with these pre-core mutants is associated with continued viral replication and liver damage, that is frequently severe. Clinical characteristics of a pre-core mutant infection are progressive liver disease, the absence of HBeAg, the presence of anti-HBe, and the presence of HBV DNA that indicates ongoing viral replication. Therefore, an assay of HBV DNA is required to diagnose pre-core mutant disease.

Chronic hepatitis as a result of pre-core HBeAg-negative HBV variants runs a different clinical course than that of the classic HBeAg-positive disease caused by wild-type HBV [Bonino et al., 1986; Hadziyannis, 1995]. In most patients, pre-core mutant-associated disease progresses to cirrhosis in a relentless, though often erratic, pattern. Multiple peaks in serum alanine amino transferase (ALT) levels and accompanying peaks of viraemia may alternate with prolonged periods of biochemical and viral quiescence over the years [Bonino et al., 1991; Hadziyannis, 1995; Dusheiko, 1999] (Fig. 1).

EPIDEMIOLOGY OF PRE-CORE MUTANT CHRONIC HEPATITIS B INFECTION

Pre-core mutant (HBeAg-negative) chronic HBV hepatitis exists world-wide, but is particularly common in Mediterranean Europe and Asia. The world-wide and regional prevalence remains to be fully established. An analysis of prevalence rates from selected studies, that included a number of differing patient populations, showed large variations between groups [Schalm et al., 1990]. This analysis showed that HBeAg-negative chronic hepatitis B (pre-core mutant HBV) accounts for 7–30% of patients with chronic HBV infection world-wide. Prevalence rates of 50–80% in the Mediterranean area (Italy, Greece and Israel) and 40–55% in Asia (Hong Kong, Taiwan and Japan) have also

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Accepted 2 February 2000

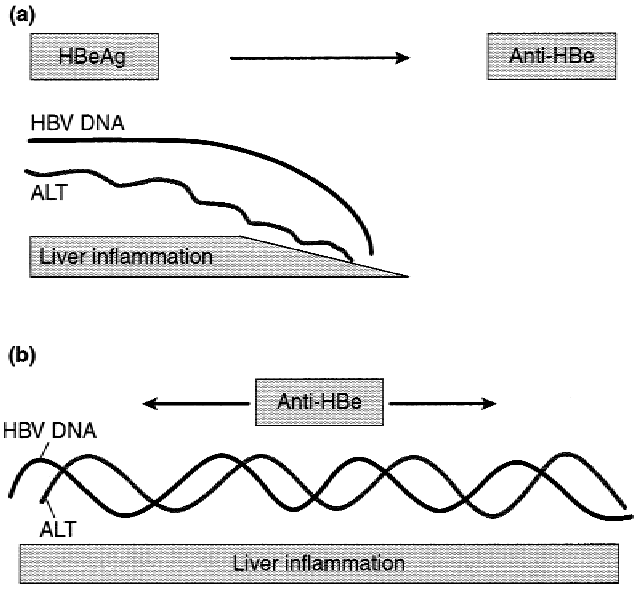

Fig. 1. (**a**) The course of chronic hepatitis B e antigen (HBeAg)-positive hepatitis B virus (HBV) infection; (**b**) the course of chronic HBeAg-negative (pre-core mutant dominant) HBV infection. ALT, alanine amino transferase; Anti-HBe, antibodies to HBeAg.

been reported [Santantonio et al., 1991; Hadziyannis, 1995; Lindh et al., 1996]. The variability in the prevalence of pre-core mutant HBV in different geographical locations may be related to the predominant genotypes of HBV circulating in each population.

Other studies of the prevalence of the pre-core mutant-associated HBV infection have indicated a more variable prevalence in Asia. In one study of Chinese patients, it was estimated that active HBV replication persists in the form of pre-core mutants in approximately 30% of Chinese anti-HBe-positive patients [Tu et al., 1997]. In Japan, one study found the prevalence of pre-core mutant HBV to be 58% (15/26) [Karasawa et al., 1997], and in another study a prevalence of 83% (51/61) was reported [Nakahori et al., 1995]. Studies in Korea indicated that 38% (21/55) of patients with chronic hepatitis B have pre-core mutant infection [Kim et al., 1993], and that pre-core mutants were detectable in 86% (50/58) of patients with chronic hepatitis B-related hepatocellular carcinoma for whom DNA sequencing was possible [Park et al., 1997]. In India, 15% (18/120) of chronic hepatitis B carriers were found to have pre-core mutant infection [Guptan et al., 1996]. These studies show that pre-core mutant infection makes a substantial contribution to hepatitis B infection across the world and that its treatment is an important component in the control of hepatitis infection and associated disease.

During the last decade the prevalence of chronic hepatitis B has decreased throughout the Mediterranean countries. Contributory factors include increased public and medical awareness of hepatitis B and its mode of transmission, reduction in family size, increased medical attention to hepatitis B surface antigen (HBsAg) carriers within a household, and the availability of vaccines [Rizzetto, 1998]. This change in the endemicity of HBV infection in the Mediterranean countries has been accompanied by a striking virological change. In Italy, 58% of 534 chronic hepatitis B patients studied over the period 1975–1985 were HBeAg-positive and 42% were HBeAg-negative (most of whom had anti-HBe) [Giusti et al., 1991]. In 1997, however, in a group of 834 carriers with similar clinical features studied in 12 Italian medical centres, the prevalence of HBeAg-positive chronic hepatitis B was only about 10%; the remaining 90% of carriers were either HBeAg-negative or anti-HBe-positive [Gaeta GB, personal communication] (Fig. 2). A similar virological shift has occurred throughout Southern Europe. This shift in virologic presentation is characterised by a change from the presence of HBeAg in the serum (i.e., infection with the wild-type HBV) to an absence of HBeAg or the presence of the homologous antibody (i.e., infection with pre-core mutant varieties) [Dusheiko, 1999]. Therefore, in Southern Europe, most chronic HBV infections are sustained currently by pre-core mutant viruses that over a period of only two decades have largely replaced the wild-type virus.

PRE-CORE MUTANT CHRONIC HEPATITIS B INFECTIONS

The predominant mutation present in HBeAg-defective HBV has been identified as a point mutation at nucleotide 1896. This mutation converts codon 28 from a tryptophan residue to a termination/STOP-codon, thus preventing the expression of the pre-C sequence and secretion of HBeAg. This leads to the phenotypic absence of HBeAg in pre-core mutant-infected individuals [Brunetto et al., 1989; Carman et al., 1989; Bonino et al., 1991]. The error-prone replication cycle generates continuously mutants of all kinds during infection. The mutants probably arise over time by positive selection exerted by the efficient elimination of HBeAg, that is a major target for activated T lymphocytes [Bonino et al., 1991]. The improvement in HBV control in Southern Europe has presumably led to a large fall in the number of new infections sustained by the wild-type HBeAg-positive HBV, with a corresponding relative increase in the residual long-standing infections that are associated with the HBeAg-negative (pre-core) mutant [Rizzetto, 1998].

Hepatitis B viruses are divided into genotypes A–F [Norder et al., 1993], that are not distributed evenly geographically. New molecular insights into the functional implications of the mutations associated with pre-core mutants provide an explanation for their geographical distribution. Genotypes D and F tolerate the 1896 mutation whereas, among the A and E genotypes, the mutation destabilises the epsilon structure that is required for viral replication, thus generating non-viable mutants [Lok et al., 1994]. The prevalence of genotype D matches the prevalence of HBeAg-negative mutants in the Mediterranean region, in contrast to the conspicuous absence of mutants in Northern Eu-

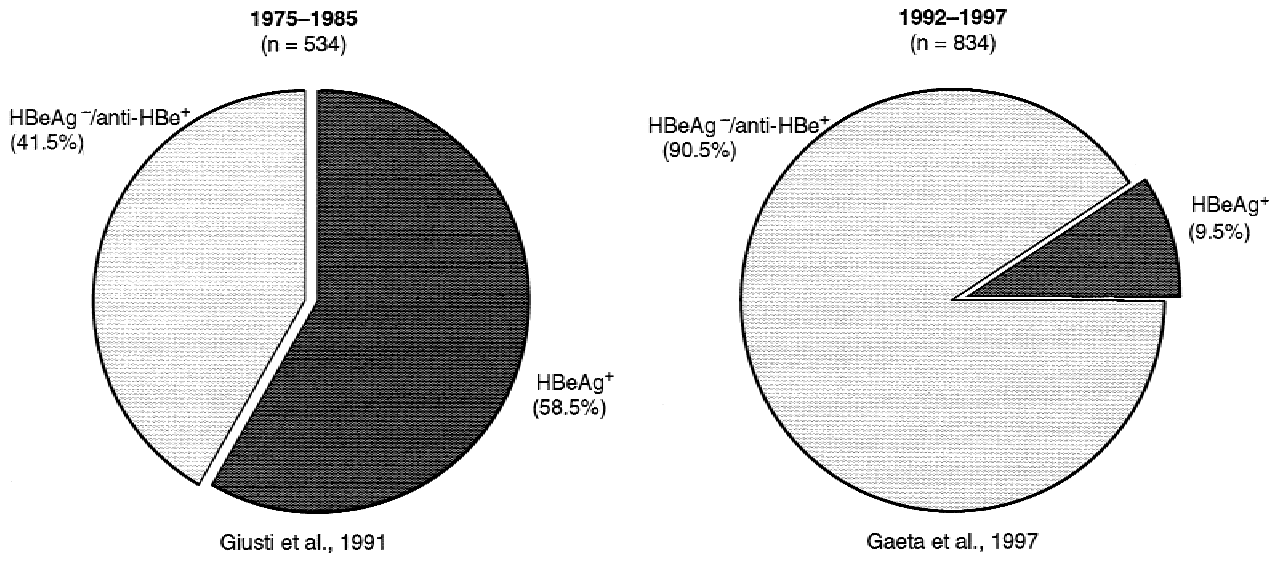

Fig. 2. The changing prevalence of hepatitis B e antigen (HBeAg)-negative chronic hepatitis B infection in Italy. Anti-HBe, antibodies to HBeAg.

rope and North America, where other genotypes are predominant [Norder et al., 1993].

As a consequence of these recent epidemiological changes, physicians in Southern Europe rarely see patients presenting with chronic HBV disease characterised by the presence of HBeAg – patients who usually have fairly mild disease and respond relatively well to therapy with interferon (IFN) alpha. Instead they are now faced with patients with longstanding anti-HBe-positive disease, who acquired their infection when HBV was hyperendemic in the Mediterranean area and have developed subsequently pre-core mutants and advanced disease. These patients present a different therapeutic challenge.

LAMIVUDINE AS A TREATMENT FOR PRE-CORE MUTANT CHRONIC HEPATITIS B

Pre-core mutant-associated disease responds poorly to IFN alpha therapy [Hadziyannis et al., 1990; Pastore et al., 1992; Brunetto et al., 1993]. Because of the urgent need for an alternative treatment, lamivudine has been investigated for the treatment of pre-core mutant chronic hepatitis B.

A placebo-controlled, randomised, double-blind study [Tassopoulos et al., 1999a] was carried out, examining patients aged 16–70 years who had been HBsAg-positive, anti-HBe-positive and HBeAg-negative for at least 6 months. Patients eligible for inclusion in the study had a serum HBV DNA concentration at screening of at least 2.5 pg/ml, and raised serum ALT concentrations (>1.5–10 × upper limit of normal) at screening as well as at least one occurrence of raised serum ALT concentration before the 3 months before screening. Within 4 weeks of the screening visit, patients were assigned randomly to receive either 100 mg lamivudine (n = 60) or placebo (n = 65) , that they took orally once daily. At Week 24, serum samples were taken and analysed for HBV DNA. At Week 26, the study was unblinded and patients who were HBV DNA-positive were withdrawn. Patients without detectable HBV DNA who were in the lamivudine group continued with the treatment up to Week 52. Those who were in the placebo group stopped treatment but remained in the study on follow-up. Patients who received lamivudine for 52 weeks have been followed up subsequently, without treatment, for 6 months [Tassopoulos et al., 1999b].

The primary efficacy end-point was loss of serum HBV DNA together with normalisation of ALT at Week 24. There were three categories of response to treatment: complete response (HBV DNA not detectable, ALT within normal limits), partial response (HBV DNA not detectable, ALT not normalised), and non-response (detectable HBV DNA). The secondary efficacy parameters included histological response from baseline to Week 52 in the lamivudine-treated patients. Biopsy specimens for histological assessment were taken at baseline (all patients) and Week 52 (patients in the lamivudine group who remained in the study). Biopsy specimens were evaluated by one histopathologist according to the Knodell Histological Activity Index (HAI) [Knodell et al., 1981].

Fifty-four patients in each treatment group were eligible for inclusion in the primary efficacy analysis. A significantly ($P < 0.001$) higher proportion of patients receiving lamivudine (63%) had a complete response at week 24 compared with patients who received placebo (6%) (Fig. 3). There was a partial response in 28% of patients in the lamivudine group and in 20% of those in the placebo group. Only 9% of patients who received lamivudine were non-responders at Week 24, compared with 74% of those who received placebo. In each of the five non-responders in the lamivudine group, serum HBV DNA concentrations were substantially

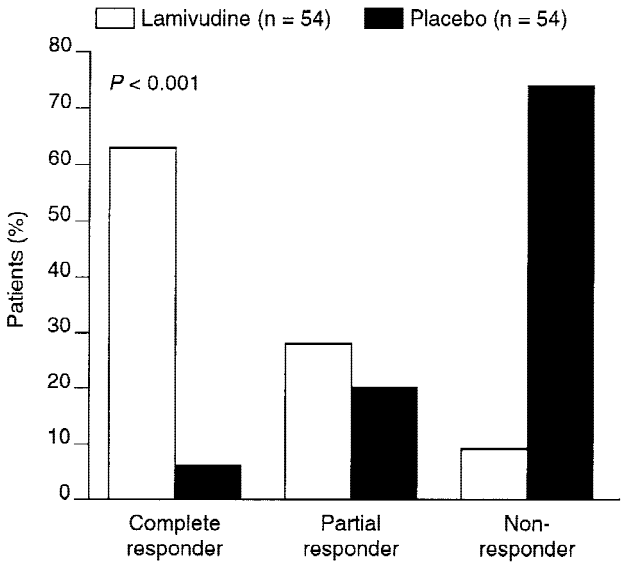

Fig. 3. The response of pre-core mutant associated chronic hepatitis B [alanine amino transferase (ALT) and hepatitis B virus (HBV) DNA] to lamivudine after 24 weeks of therapy. Complete response: HBV DNA not detectable, ALT within normal limits; partial response: HBV DNA not detectable, ALT not normalised; non-response: detectable HBV DNA. Reproduced with permission from Tassopoulos et al., 1999a.

lower than at baseline. At Week 52 the proportion of patients with a complete response remained high (65%).

At Week 52, there was measurable histological improvement (≥2 point reduction in the Knodell necroinflammatory score) in 60% of patients treated with lamivudine who had adequate biopsy specimens available (n = 42). Histological deterioration occurred in only 12% of the lamivudine group. A ranked assessment of pre- and post-treatment biopsy pairs (n = 44) showed that fibrosis was improved in 11% of patients, there was no change in 86% of patients and fibrosis worsened in 2% of patients.

During Weeks 0 to 26, the incidence of adverse events and laboratory abnormalities was generally similar in both treatment groups. The incidence of grade 3/grade 4 ALT abnormalities was higher in the placebo group (12%; 8/65 patients) than in the lamivudine group (3%; 2/60 patients), but in none of the patients was the increase in ALT associated with a significant elevation in serum bilirubin.

The incidence of YMDD (tyrosine-methionine-aspartate-aspartate amino acid motif of HBV polymerase) variant HBV was evaluated in all patients for whom serum samples were available at Weeks 26 and 52. At Week 26, one patient out of 53 in the lamivudine group (<2%) had YMDD variant HBV. At Week 52, 11 out of 41 patients (27%) receiving lamivudine had YMDD variant HBV. These 11 patients had a median HBV DNA of 320 pg/ml at baseline, less than 2.5 pg/ml at week 52 and 32 pg/ml at week 76 (coinciding with a post-treatment return of wild-type virus) [Tassopoulos et al., 1999a, 1999b]. Only two of the 11 patients with YMDD variant HBV had increased concentrations of serum ALT at Week 52.

After 24 weeks of follow-up (Week 76 from initiation of therapy), 11% (16/54) of the patients who had received lamivudine for 52 weeks remained HBV DNA-negative and 17% (9/54) had serum ALT concentrations within the normal range [Tassopoulos et al., 1999b]. Only 11% (6/54), however, had both normal ALT and undetectable HBV DNA. Median serum HBV DNA concentration was less than 2.5 pg/ml at Weeks 52 and 76, compared with 255 pg/ml at baseline. The incidence of post-treatment ALT elevations that were greater than 3 times the ULN was 12% (seven out of 58 patients). None of these ALT elevations was associated with significant elevations in bilirubin.

A compassionate use programme for lamivudine has involved more than 300 patients with detectable (<5 pg/ml) serum HBV DNA at baseline. Preliminary data, after median exposure to lamivudine lasting 8.4 months (range 0–37 months), show that 78% (198/255) of patients remained HBV DNA-negative at last visit. Median serum concentration of ALT decreased from 98 IU/litre (n = 336) at baseline to 33 IU/litre (n = 269) at last visit.

CONCLUSIONS

These data show that lamivudine has a profound and prolonged suppressive effect on the HBV pre-core mutants and is, therefore, an effective treatment for patients with pre-core mutant chronic hepatitis B. Inhibition of HBV replication is accompanied by a significant normalisation of serum ALT levels and an improvement in liver disease. YMDD variants are selected at similar rates to those reported during treatment of HBeAg-positive (wild-type) disease in western patient populations. Lamivudine is the first effective oral therapy for chronic hepatitis B patients with viral replication and liver disease. Most studies have investigated lamivudine in cases of HBeAg-positive (wild-type) disease, but this paper shows that lamivudine is suitable for a wide range of patients with chronic hepatitis B, including those with pre-core mutant HBV infection.

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